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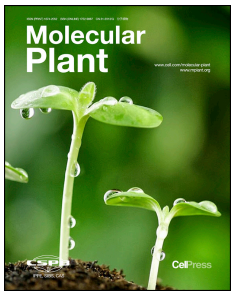

Multicellular systems biology: quantifying cellular patterning and function in plant organs using network science

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TITLE

Multicellular systems biology: quantifying cellular patterning and function in plant organs using network science

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ABSTRACT

Organ function is at least partially shaped and constrained by the organization of their constituent cells. Extensive investigation has revealed mechanisms explaining how these patterns are generated, with less being known about their functional relevance. In this paper, a methodology to discretize and quantitatively analyze cellular patterning is described. By performing global organ-scale cellular interaction mapping, the organization of cells can be extracted and analyzed using network science. This provides a means to take the developmental analysis of cellular organization in complex organisms beyond qualitative descriptions, and provides data-driven approaches to inferring cellular function. The bridging of a structure-function relationship in hypocotyl epidermal cell patterning through global topological analysis provides support for this approach. The analysis of cellular topologies from patterning mutants further enables the contribution of gene activity towards the organizational properties of tissues to be linked, bridging molecular and tissue scales. This systems-based approach to investigate multicellular complexity paves the way to uncovering the principles of complex organ design, and achieving predictive genotype-phenotype mapping.

KEYWORDS

Organ, network, connectivity, transport, tissue topology, connectome

Introduction

Organs are self-contained collections of interacting cells that perform a function that individual cells cannot (Bonner, 1988). Through the process of emergence, these multicellular consortia give rise to complex life (Solé and Goodwin, 2000). What organs are capable of achieving is at least partially shaped and constrained by structure-function relationships at the cellular level such that their organization impacts their collective performance (Ollé-Vila et al., 2016; Thompson, 1942).

Previous studies have identified principles and mechanisms by which patterns are created in plants, including the contribution of each genetic pathways and biophysical forces (Gaillochet et al., 2015; Hamant et al., 2008). The use of genetic mutant screens have identified molecular agents that underpin the active control of specific cell divisions that give rise to cell arrangements, and provide key insight into how local arrangements of cells are created (DiLaurenzio et al., 1996; Hardtke and Berleth, 1998).

The immobility of cells in plant tissues makes the combination of the control of the cell cycle, and orientation of the division plane, the key factors defining cellular organization (Meyerowitz, 1997). Given these constraints, asymmetric divisions are sufficient to generate novel arrangements (De Smet and Beeckman, 2011; Dong et al., 2009; Smith, 2001). Several examples of this include the formation of stomata (Raissig et al., 2017), the creation hypophysis during embryo development (Schlereth et al., 2010), and cell divisions leading to cortical and endodermal cell layers during root development (DiLaurenzio et al., 1996).

These studies provide detailed and mechanistic insights as to how local arrangements of cells are generated. How these groups of cells come together to form a global integrated system of interacting cells at the organ scale, is less well understood. The functional consequences cellular organization has on organ function and structure-function relationships at this scale also represents a knowledge gap. In order to address these questions, a discrete and quantitative approach to extract and analyze cellular architecture is required.

Anatomical analyses have historically provided largely qualitative descriptions of tissues, with quantitative approaches to analyzing structure rapidly being developed. Methods to analyze cell shape (Pincus and Theriot, 2007; Sánchez-Corrales et al., 2018), curvature across organs (Kierzkowski et al., 2012), and root systems (Fang et al., 2013) in quantitative ways have been applied previously. Gaps remain in understanding organ level organizational properties at a cellular level.

An approach that has been transformative to understanding nervous system structure and function is that of cellular interaction mapping (Ramon y Cajal, 1911) and “connectomics”

(Sporns et al., 2005). By performing cellular interaction mapping of neurons, the organizational properties of nervous systems, and function of individual neurons, has been predicted (Chalfie et al., 1985).

In this article an extension of connectionist approaches to analyzing cellular organization is proposed. This creates for a “systems biology of the organ”, opening the door to a host of investigative opportunities provided by network and complexity science.

The origins of cellular interaction mapping

The advent of cellular interaction mapping can be traced to Ramon y Cajal (Ramon y Cajal, 1911). By staining tissues with a Golgi stain and performing light microscopy, Cajal was able to visualize the connections between individual neurons in various animal tissues. A more systematic approach was later taken to comprehensively map each of the 302 neurons and their connections within the worm *C. elegans* (White et al., 1986). This resulted in the production of the first “wiring diagram”, a network describing global neuronal connectivity (connectome) (Sporns et al., 2005). In the case of such an interaction network, cells are represented by nodes, and their physical associations as edges. The network-based analyses of this dataset has proven sufficient to predict the function of individual neurons in touch sensitivity (Chalfie et al., 1985), and motor control (Yan et al., 2017). The only other completed neuronal connectome to date has come from the sea squirt, 30 years after that of *C. elegans*, and contains 177 neurons (Ryan et al., 2016).

The topological analysis of connectomes has been sufficient to predict the function of individual cells in nervous systems in *C. elegans*, demonstrating the ability to bridge structure-function relationships in cellular consortia using network science. While a neuron is one of many cell types which contribute to the construction of complex animals, many organisms including plants, don’t have nervous systems, and consist of collections of diverse cells types upon which information may be processed (Baluška and Levin, 2016; Bassel, 2018). The investigation of relationships between cell organization and function in non-neuronal systems remains largely unexplored.

Organs as integrated systems of interacting cells

Communication between cells underpins the emergent behaviour of organs (Solé and Goodwin, 2000). Multicellular plants may therefore be viewed as integrated multicellular transport systems. In these systems, mobile information may be any developmentally instructive molecule, which can include mRNA (Lucas et al., 1995), miRNA (Carlsbecker et al., 2010),

proteins (Nakajima et al., 2001), ions (Knight et al., 1991), hormones (Swarup et al., 2001; Tal et al., 2016), and peptides (Ogawa et al., 2008). Networks describing the connectivity between cells capture the possible routes of information movement across the organ.

Symplastic connections between cells, mediated by plasmodesmata, represent a primary means of information movement between cells (Brunkard and Zambryski, 2017; Fitzgibbon et al., 2013; Lucas and Lee, 2004). Cells also communicate through specific membrane-bound transporters, and their intercellular space termed the apoplast (Blilou et al., 2005). In these latter cases, the proximity between adjacent cells plays a key role in determining the destination of extracellular mobile information, making cellular proximity and association relevant in these instances as well.

Abstraction of plant organs into cellular interaction networks

In order to identify physical associations between cells in plant organs, image-based approaches may be applied (Figure 1). Imaging techniques involving clarification of fixed tissue provide a means to perform whole organ cellular resolution imaging that is otherwise limited by optical aberration of laser light (Kurihara et al., 2015; Palmer et al., 2015; Truernit et al., 2008). These techniques enable all cells in entire organs to be digitally captured in 3D from z-stacks using confocal microscopy. In contrast to neuronal connectomics which focuses on a single cell type, this approach focuses on all cells within the organ, providing a comprehensive approach to understanding global structure of these multicellular systems.

The abstraction of these image volumes into networks describing cellular interactions requires computational image analysis (Bassel and Smith, 2016; Roeder et al., 2012; Roeder et al., 2011). Cells are segmented in 3D, and surfaces are defined using polygonal meshes (Cuno et al., 2004), leading to the identification of nodes in the network. Cell surfaces which are in physical association with one another are identified, enabling the establishment of edges (Montenegro-Johnson et al., 2015). The geometric size of these shared intercellular interfaces can be quantified based on the amount of shared interface between cell pairs. The output of this analysis comes in the form of a text file describing the pairwise interactions between cells in the organ analyzed.

The physical nature of this image analysis provides robust datasets, which are both accurate and reproducible (Jackson et al., 2017b; Yoshida et al., 2014). In contrast to other biological interaction network data which are often incomplete and subject to inaccuracies due to false positives and negatives (Von Mering et al., 2002), cellular interactome datasets capture all cells and interactions with high confidence due to the physical nature of the measurements. The

complete nature of these datasets (capturing the entire system) further provides powerful opportunities to perform quantitative network-based analyses to explore the system-wide properties with high confidence given no aspects of the system are missing. The further identification of cell types within cellular representations of organs, using for example 3DCellAtlas (Montenegro-Johnson et al., 2015), enables cell type specific topological analyses to be performed (Jackson et al., 2017b).

Topological analysis of plant cellular interaction networks

Unlike animal tissues where cells move around, cells in plant organs are fixed in their position (Coen et al., 2004). This renders cellular organization topologically invariant, and simplifies the analysis of these static cellular arrangements. Cells are typically tessellated within plant organs, with some notable exceptions such as the leaf, where air spaces are formed. The lattice-like nature of these cellular arrangements provides templates upon which molecular events take place.

The topological analysis of networks describing plant cellular organization can be performed at each local and global scales (Jackson et al., 2017a; Jackson et al., 2017b). The simplest local question that can be asked is how many direct neighbours a cell has. In network terminology, this is termed degree (Barabási, 2016; Newman, 2010), and measures how much local influence an individual cell has at a given location within an organ (Figure 2B). The degree of cells has been explored in the context of animal and plant epithelia previously, and described in terms of polygons (Carter et al., 2017; Gibson et al., 2006; Gibson et al., 2011; Sahlin and Jönsson, 2010). The polygonal count of these cells is a readout of their number of neighbours, and was demonstrated to converge on a distribution centering at the number six. The cell cleavage plane in the *Drosophila* imaginal wing disc and cucumber shoot apical meristem (SAM) was further shown to have a bias towards intersecting the neighbouring cell having the lowest degree, thus increasing the number of neighbours of this adjacent cell (Gibson et al., 2011). It was proposed that this generative process leads to the maintenance of topological order in these tissues.

While informative, degree does not capture the role of cells within the broader context of multicellular systems (Barabási, 2016). In light of the immobility and spatial constraints on these multicellular transport networks, the property of path length represents a biologically significant topological feature (Barthélemy, 2011). On a local scale, cells are only able to directly communicate with their immediate neighbours. On the global scale, cells are indirectly in contact

with one another through other intermediary cells, a property which may play a functional role in organ function.

In traversing a network, optimal routing is to follow a shortest path between two nodes, representing the minimum number of edges travelled (Barabási, 2016; Newman, 2010). The same applies to plant organs where molecular movement within a cytoplasm is less costly than passing through cell interfaces. Cells which lie upon shortest paths between other pairs of cells are therefore able to control the flow of information across an organ.

Network centrality measures have been developed which are capable of identifying nodes that lie upon a greater number of shortest paths between other pairs of nodes. Betweenness centrality (BC) uses prior knowledge of the complete network to calculate the shortest paths between all pairs of nodes (Freeman, 1977) (Figure 2C). Cells which lie upon a greater number of shortest paths between other cell pairs have a higher BC.

Random Walk Centrality (RWC) does not use prior knowledge of the system to calculate shortest paths. Source nodes send out many random walkers and track which nodes are traversed until they reach their destination (Newman, 2005) (Figure 2D). Nodes which are traversed more frequently are given a greater RWC. Very large number of random walkers are required to identify high RWC paths as a minority of individual agents follow near-optimal routes due to their random motion.

In the case of both BC and RWC, having a greater value increases the ability of a cell to control information flow across an organ as it identifies cells lying upon a greater number of shortest paths. This represents a biologically relevant property of organs in light of the system-wide communication which takes place.

The application of global topological analyses including BC and RWC to plant connectomes is appropriate when these systems are fully represented, with all cells and interactions present. The ability to derive meaningful results from partial or inaccurate datasets using global calculations is limited given the global reach of these measures.

The identification of a shortest path in a network without prior knowledge of its topology represents a complex logistical problem. This is equivalent to identifying optimal routes in navigating a city without a map. This scenario is encountered by tissues, as they do not have information as to where all other cells are located within an organ. A recently developed measure named Navigation Centrality (NC) (Seguin et al., 2018), analogous to Greedy Navigation (Muscoloni and Cannistraci, 2019; Muscoloni et al., 2017) provides a simple propagation rule that is able to identify near optimal shortest paths using only local information. By integrating the geometric information as to how nodes are embedded in space, progressive

steps following a gradient to a destination is followed (Figure 2E). NC therefore provides a biologically realistic calculation to identify optimal routes which are not provided by other measures such as BC and RWC. This centrality has yet to be applied to multicellular tissues outside of the nervous system, yet provides a promising approach in light of the nature by which the calculation is performed.

Another biologically relevant consideration in the analysis of multicellular tissues is the efficiency by which they can exchange information (Barabási, 2016). This may be considered at each the local and global scales (Latora and Marchiori, 2001). The global measure considers how efficient a whole system is at transmitting information, while local efficiency quantifies the resilience of this information movement on a small scale in the face of individual failures. A tradeoff between global and local efficiency and the optimization between each of these in different contexts represents an important design feature in diverse transport systems, and provides another promising approach to understanding tissue architecture.

Several caveats in the analysis of cellular interaction network datasets must be considered. Input datasets need to be carefully curated to ensure their maximal accuracy and completeness. The lattice-like nature of these networks and the small number of nodes and edges lead small inaccuracies to have large consequences on subsequent analyses. In order to achieve robust global path length-based analyses, fully accurate networks are required (Barthélemy, 2011).

A second caveat lies with the nature of topological analyses performed. It necessary to consider the calculation that is made and its relevance to biology. How a cell calculates its number of neighbours remains an outstanding question (Gibson and Gibson, 2009), while centralities such as BC are not biologically feasible as plants lack maps of their cellular organization (Baluška and Levin, 2016). Despite the biological challenges associated with both degree and BC, their measurement still provides important insight into the organization of cells in organ, while the manner in which they are interpreted requires the appropriate caution.

Structural and functional networks

The capture and abstraction of global cellular interactions into networks is analogous to the creation of a map describing a transport system such as a rail network (Barthélemy, 2011). All the routes of possible movement are described by the representation.

In the context of cellular interactomes, these are termed *structural networks*, and describe the possible routes of information flow across an organ (Figure 3A) (Bullmore and Sporns, 2009). Following this rail system analogy, these maps do not provide a schedule indicating the

timetable or speed of the trains. In order to achieve this, functional annotation of the map with additional information is required.

A *functional network* is a structural network that has been annotated with additional dimensions of data (Bullmore and Sporns, 2009) (Figures 3B-C). In the case of a plant connectome, functional annotation could include information relating to either the nodes (cells) (Figure 3B) or the edges (interfaces) (Figure 3C), or both, depending on what is being investigated.

Node annotation may include different data types, including for example the abundance of a protein within a cell, or the intensity of a biosensor. The application of these data to the network involves the additional of a value to an individual node (Figure 3B).

Edge annotation may include the presence and/or abundance of a transporter, the abundance of plasmodesmata/pit fields on cell interfaces, or the size of cell interfaces. Functional annotation of plasmodesmata aperture can also be evaluated by measuring the rates of movement of fluorescent molecules between adjacent cells (Gerlitz et al., 2018). The application of these data to a network data structure involves the addition of values to edges (Figure 3C).

Understanding each the abundance and aperture of plasmodesmata on intercellular interfaces is central to understanding system-wide organ communication. While structural templates provide routes of possible information flow, functional annotation represents that which is observed to occur. With this in mind, two cells which are physically associated are not necessarily communicating. In order for that to occur there both needs to be plasmodesmata present, and they also need to be open. Symplastic connections are dynamic and change across plant development (Rinne et al., 2001; Rinne et al., 2011), indicating that functional annotation itself is temporal in nature (Holme and Saramäki, 2012).

The functional annotation of plant connectomes may be greatly aided by techniques involving the gel-based embedding of tissues including PEA-CLARITY (Palmer et al., 2015). This technique enables the repeated localization of molecular components within tissues through repeated rounds whole mount *in situ* hybridization using antibodies and/or oligonucleotides. Samples may repeatedly be stripped and reprobed, analogous to a western blot membrane. This provides a highly multidimensional functional annotation with multiple rounds of probing of the same sample, and transcends the limits imposed by resolving individual fluorophores using confocal microscopy.

With the functional annotation of networks comes additional values which can be integrated into centrality measurements. These centralities are in turn calculated using these “weighted” values, and simultaneously integrate both the organization of cells and their functional properties in the outputs.

Case study 1: the plant hypocotyl

Global structural cellular interaction mapping has been applied to understanding the organization of cells in the plant hypocotyl (Jackson et al., 2017b). Following the germination of the embryo, cells in the hypocotyl elongate to promote seedling development, in the absence of cell divisions (Gendreau et al., 1997; Sliwinska et al., 2009). Similar to the root, cells in the hypocotyl have a radial and modular organization (Figure 4A). In both organs, two cell types are present in the epidermis: trichoblasts which produce hairs which promote nutrient uptake, and atrichoblasts which are adjacent to trichoblasts but do not produce hairs. Studies have uncovered detailed genetic mechanisms that lead to the formation of these two cell types, describing how epidermal patterning is generated (Dolan, 2005; Duckett et al., 1994). The functional relevance of the stereotyped pattern of these two cell types is less well understood.

An attempt to bridge structure and function in epidermal cell organization was undertaken using a connectionist approach (Jackson et al., 2017b). Whole mount 3D imaging of fixed samples resulted in the capture and discretization of cellular connectivity in this organ. Individual cell types were identified (Figure 4A) (Montenegro-Johnson et al., 2015) in quadruplicate biological replicates enabling cell type specific topological analyses of patterning to be performed together with statistical analyses.

In the *Arabidopsis* hypocotyl epidermis it was found that trichoblasts had more neighbours (higher degree) than atrichoblasts (Figure 4B). Contributing to this is their position above two underlying cortical cells, in contrast to atrichoblasts which are above one (Figure 4A). Path length calculation revealed that atrichoblasts have a higher BC than trichoblasts (Figure 4C). Despite having fewer neighbours, atrichoblasts lie upon a greater number of shortest paths than their counterparts. This represents a non-intuitive higher-order property of epidermal patterning in the hypocotyl, and the presence of conduits of reduced path length along the longitudinal axis of this organ.

The functional relevance of these conduits of reduced path length was examined by placing seedlings onto media containing fluorescein and imaging using confocal microscopy to identify where it moves (Duran-Nebreda and Bassel, 2017b). Bulk molecular movement of the fluorescein molecule followed the identified shortest paths through the hypocotyl via the atrichoblast cell type (Figure 4D). Given a map of global cellular organization, BC is therefore sufficient as a topological proxy to predict molecular movement at single cell resolution through this complex multicellular plant system. The means by which this shortest path calculation is

performed in the biological context remains enigmatic, with Navigation Centrality providing a promising approach to understand how this may be achieved (Seguin et al., 2018).

These observations provide a potential link between structure and function in epidermal cell patterning in the hypocotyl in *Arabidopsis*. Each hair cell promotes solute uptake, and is flanked by 2 non-hair cells. The higher-order organization of non-hair cells facilitates them for the optimized longitudinal movement of molecules. A division of labour is therefore implemented whereby hair cells perform nutrient uptake, while non-hair cells aid transport (Figure 4E). This enables intracellular solute concentration to be kept low in trichoblast cells facilitating their uptake function, while providing optimized conduits for molecular movement on the surface of the organ. In this example, the analysis of a structural network alone is sufficient to predict cell function in the hypocotyl epidermis.

Analyses of biological quadruplicates from 3 different *Arabidopsis* ecotypes identified statistically significant genotype-specific differences epidermal path length (Jackson et al., 2017b). This demonstrates the presence of conserved emergent patterning properties at each the local and global scales in the hypocotyl, and the ability to reliably perform quantitative analyses of cellular global organization in plants using connectionist approaches. It further highlights the presence of patterning plasticity within the epidermis of the *Arabidopsis* hypocotyl in different genetic backgrounds which have higher-order organizational consequences. This plasticity may provide a means for modulating adaptive fitness at the organ design level in light of these structure-function relationships.

Case study 2: the developing plant embryo

Early embryo development in *Arabidopsis* was also investigated using a connectionist approach previously (Yoshida et al., 2014). The topological analysis of the 16 cell embryo was performed with a view to understanding how pairs of cells are connected to one another. To address this, the frequency of shared neighbours, the number of shared 1st degree connections between pairs of nodes (Assenov et al., 2007), was determined. This represents a mesoscopic analysis of the connectedness of a system.

Wild type embryos (Figure 5A) were compared with transgenic individuals expressing the *IAA12/BODENLOS* (*BDL*) gene (Figure 5B), which is a repressor of the *AUXIN RESPONSE FACTOR5* named *MONOPTEROS* (*MP*) (Hamann et al., 2002). *MP* has been demonstrated to carry defects in embryo patterning (Hardtke and Berleth, 1998), and plays a role in mediating the contribution of the hormone auxin towards creating this structure. *BDL* was placed under the control of the *RPS5A* promoter (*RPS5A::bdl*), supporting high level expression in the embryo.

The number of shared neighours in the transgenic *RPS5A::bdl* expressing line was greater than that of the wild type embryo, indicating an overall increase in the connectedness of this system (Figure 5C). This provided a quantitative link between auxin signaling mediated by *MP* and the organization of cells in the developing embryo. The analysis of biological triplicate samples supported the robustness these mesoscale patterning properties in this tissue.

Case study 3: the plant shoot apical meristem

A recent study topologically investigated the organization of cells within the SAM at both local and global scales (Figure 6A) (Jackson et al., 2019). Time lapse live imaging was performed to examine the relationship between cellular organization and the control of the cell cycle. Cellular connectivity networks of cells in the SAM were extracted from images, and the topological dynamics of this network was established using image registration and lineage tracking (de Reuille et al., 2015; Fernandez et al., 2010).

Cells were separated into 3 classes: those which do not divide, cells which will divide, and daughter cells following divisions (Figure 6B). The volume of cells was capable of discriminating between these size classes, consistent with previous reports describing a size control mechanism for cells in the SAM (Jones et al., 2017; Willis et al., 2016). While cell shape (anisotropy) did not discriminate whether or not a cell would divide, the number of neighbours (degree) did, along with the number of shortest paths a cell lies upon (BC and RWC) (Figure 6B). These data demonstrated cells in the SAM to be undergoing both geometric and topological cycling.

These observations further suggested that topology can be used to identify when a cell will divide, as cells that lie upon an increasing number of shortest paths are more likely to undergo a division. Following this division, the number of shortest paths the daughter cells is limited (Figure 6B), opening the possibility that the placement of a cell division plane may be predicted based on the global topology of the tissue.

Computational analysis of cell division planes based on the minimization of the degree and RWC of daughter cells was performed, along with the local geometric rule from Errera that divides a cell in half using the shortest possible wall which passes through the middle (Besson and Dumais, 2011; Errera, 1888) (Figure 6C). The local geometric rule of Errera and the global topological RWC Minimizing rule were found to largely both predict the same division plane, and show similar deviations from observed division planes (Figure 6D). The results collectively suggested that the local shape of a cell within the SAM predisposes it for a shortest wall division plane that also satisfies the minimization of RWC in the daughter cell

Intercellular interactions play a key role in the control of cell shape in plant tissues with cells being connected through shared cell walls (Coen et al., 2004). The microtubule severing protein *KATANIN1* (*KTN1*) has been demonstrated to mediate mechanical interactions between cells and regulate morphogenesis of the SAM (Uyttewaal et al., 2012). Geometric analysis of cell shape in the *ktn1* SAM demonstrated these to be more anisotropic that their counterparts in the wild type (Figure 6G) which in turn led to cells lying upon more shorter paths, based on increased RWC (Figure 6H). Mechanical interactions between cells therefore generate the cell shapes required for a local shortest wall division that in turn leads to the minimization of RWC in their cells (Figure 6I).

This mechanical feedback onto cell geometry and emergent generation of a tissue with minimized RWC identifies how local rules can lead to global properties. It further provides an example of complexity for free, whereby an emergent global system feature arises from simple local interactions. This example of emergent global topological order in the *Arabidopsis* SAM may extend to other tissue contexts, where division rules may either increase or limit the path length the daughter cells lie upon following their division.

The function implication of the minimization of RWC (maximization of path lengths traversed) across the SAM represents an optimization for the robustness of the system (increased local efficiency) at the cost communication speed (global efficiency), as no individual cells lie in privileged positions to facilitate rapid communication across the tissue. This may in turn impact the behaviour of this multicellular system in the face of perturbation, or internal failure. Topological homogeneity in the SAM also correlated with robustness in phylotaxis, as perturbations were observed in the *ktn1* mutant.

Bridging molecular and cellular scales

A quantitative approach to the analysis of cellular organization provides the opportunity to identify the consequences of altered arrangements in patterning mutants at local and global scales. In instances where the gene responsible for changes in cellular patterning is known, this represents a quantitative bridging of the molecular and cellular scales of plant development (Duran-Nebreda and Bassel, 2017a).

Examples of this have been described above, where a link between auxin-mediated signaling and connectivity in the 16 cell *Arabidopsis* embryo was identified (Figure 5C) (Yoshida et al., 2014), and mechanical feedbacks mediated by *KATANIN1* control cell shape and the path length upon which cells lie in the SAM (Figures 6G-I) (Jackson et al., 2019). These examples

provide quantitative links between genetic agents and the cellular configurations they contribute towards generating.

Ecotype-specific differences in the *Arabidopsis* hypocotyl were also identified with respect to the path length upon which epidermal cells lie (Jackson et al., 2017b). The functional relevance of these differences was demonstrated through the use of fluorescein transport assays which identified the preferential movement of molecules along cells lying upon shortest paths in this tissue. This relationship was faithfully followed across ecotypes having divergent path length differences between epidermal cell types.

Further quantitative analysis of altered cellular configurations were performed using *Arabidopsis* hypocotyls and the patterning mutants *CYCLIN DEPENDENT KINASEA1;1* (*CDKA1;1*) (Dissmeyer et al., 2009) and *MP* (Schlereth et al., 2010). *CDKA1;1* was found to have equal BC between hair and non-hair cells, while the BC of both epidermal cells types *MP* is significantly greater than the equivalent wild-type.

Genotype-phenotype mapping and meso scale analyses

The ability to map genotype to phenotype represents a grand challenge in biology. The measurement of each genotype through sequencing, and downstream macro outputs through phenotyping, enables links between the molecular and organismal scales (Atwell et al., 2010). Our ability to do this in a predictive fashion remains limited, and may be due to gaps in our understanding as to the mechanistic basis by which genetic changes lead to phenotypic consequences. One means to bridge this gap is through the study of the “meso” scales of development, representing the events which occur between genotype and phenotype. These include, but are not limited to, intracellular behaviour, cellular organization, inter-organ communication, and how each of these in turn interact with the environment.

A quantitative view of organ architecture, and the pursuit of investigation to understand the functional consequences of cellular configurations, may contribute towards the bridging of scales and predictive genotype-phenotype mapping. An example of this potential is provided by the identification of ecotype-specific differences in epidermal patterning across ecotypes of the *Arabidopsis* hypocotyl (Jackson et al., 2017b). These differences had consequences in terms of the bulk movement of small molecules, and suggests the higher order properties of cellular organization may represent an axis upon which natural selection acts to optimize plant fitness. Further work in this area is required to establish the extent to which this occurs in different organs and species.

Single cell sequencing and organ topology

The ability to sequence individual cells across whole organs is providing unparalleled insight into the heterogeneity of gene expression within individual cell types, and the developmental trajectories they take as they acquire their identity (Birnbaum, 2018). While powerful, these approaches require the dissociation of tissue to isolate single cells for sequencing. If one seeks to understand how cells come together to create a functional integrated organ system, this loss of positional information and their relationships presents a boundary to achieving a multidimensional understanding.

The ability to perform single cell sequencing on intact tissue where relationships between cells are preserved would reconcile this gap. This was recently achieved in moss through the use of microcapillary manipulation (Kubo et al., 2018). Further technological advances promise to bridge this gap, including the integration of landmark genes (Halpern et al., 2017).

Towards the principles of organ design

The ability to abstract and discretize patterning into networks enables quantitative comparisons between diverse genotypes and species to be performed (Avena-Koenigsberger et al., 2015). The topological analysis of these distinct datasets coupled with statistical analyses may lead to the identification of shared and divergent properties in organ design, paving the way for an understanding of the principles of cellular architecture. Elucidating how cells come together to form organs, and how those arrangements shape and constrain tissue function is central to understanding multicellular complexity. Identifying properties that emerge “for free” by virtue of cells being embedded in space, versus the mechanisms that underpin deviations from these default configurations, represent key objectives in understanding multicellular development and realizing rational morphogenetic engineering (Doursat et al., 2012; Solé et al., 2018).

Concluding remarks

The application of connectionist approaches holds promise for plant developmental biology. The construction of whole organ structural networks has begun, and paves the way for their functional annotation by the collective efforts of the community. In this way, the manner by which cells and molecules interact to create an integrated system will increase our understanding of plant development. The bridging of scales from molecules to organs may facilitate quantitative genotype-phenotype mapping. The translational promise of this approach lies with its application to crop species, to which technical boundaries do not limit this extension.

By understanding the context in which genetic programs act and how they emerge to create phenotypes, rational and predictable crop engineering may be achieved.

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TABLES

Method	Marker Visualization	Number of channels that can be visualized	Vital Marker Visualization	Subcellular resolution	Reference
mPA-PI	GUS	1	No	No	(Truernit et al., 2008)
ClearSee	Fluorescent	3-4	Yes	Yes	(Kurihara et al., 2015)
PEA-CLARITY	Fluorescent	24+	Yes	Yes	(Palmer et al., 2015)

Table 1. Summary of whole mount 3D imaging techniques for generating organ-wide cellular resolution images.

FIGURE and TABLE LEGENDS

Table 1. Summary of whole mount 3D imaging techniques for generating organ-wide cellular resolution images.

Figure 1. Workflow used to generate and analyse plant cellular interaction networks.

Figure 2. Schematic illustrating topological analyses of cellular interaction networks. (A) Extraction of a connectivity network from a hypothetical network. (B) Degree is the number of neighbours a cell has. (C) Betweenness centrality (BC) uses prior knowledge of the network to find which nodes lie upon shortest paths between all other pairs of nodes. (D) Random Walk Centrality (RWC) uses multiple random walkers to identify which cells lie upon shortest paths between pairs of nodes. This does not use prior knowledge of the network to identify cells lying upon shortest paths. (E) Navigation Centrality (NC) identifies near optimal shortest paths by using local knowledge of a network while following a gradient to a destination node.

Figure 3. Illustrations highlighting differences between structural and functional networks. (A) Structural network of a hypothetical tissue. (B) Functional annotation of cells (nodes) in the network from (A) with the abundance of a cellular factor. The greyscale is proportional to the abundance of this hypothetical component, indicated by the scale bar to the right having

arbitrary units. (C) Functional annotation of cell interfaces (edges) using the size of cell interfaces as a weighting. The size of the line width is proportional to the value.

Figure 4. Topological analysis of cellular organization in the *Arabidopsis* hypocotyl. (A) Annotation of cell types. (B) False colouring of cell degree. (C) False colouring of cell BC. (D) Concentration of fluorescein in distinct epidermal cell types of the hypocotyl. False colouring shows the relative concentration of fluorescence in each atrichoblast (a) and trichoblast (t) cells. (E) Model illustrating the division of labour between cell types in the hypocotyl epidermis. Large red arrows indicate the entry of solutes through hair cells (t). Small red arrows indicate the movement of solutes into adjacent non-hair cells (a) and their longitudinal movement is depicted by orange arrows.

Figure 5. Topological analysis of cellular organization in the 16 cell *Arabidopsis* embryo. (A) Connectivity network of the wild type 16 cell embryo and (B) the *RPS5A::bdl* expressing embryo. Cells (nodes) on the outside of the embryo are coloured green and those within the embryo yellow. The hypohysis is red and supensor is in cyan. (C) Frequency distribution of shared neighbours between the wild type and *RPS5A::bdl* expressing embryo. Error bars represent the standard deviation of biological triplicates.

Figure 6. Topological analysis of the *Arabidopsis* SAM. (A) Confocal stack of the SAM and extraction of a cellular connectivity network of the central region. (B) Geometric and topological cycling of cells which do not divide, cells which will divide, and cells which have divided in the SAM. (C) Computational prediction of cell division planes based on a local geometric rule (Errera), a rule which minimizes the degree of daughter cells, and a rule which minimizes the RWC of daughter cells. (D) Degree deviation from the observed cell division plane for each rule tested in (C). (E) Confocal image of the surface of a wild type SAM and (F) *ktn1* SAM. (G) Frequency distribution of cell anisotropy in the cells of the wild type and *ktn1* SAM. (H) Same as (G) for the RWC of cells. (I) Model describing emergence of global order in the SAM from the sensing of intercellular interactions by the cytoskeleton to minimization of RWC across the system.

of intercellular interactions by the cytoskeleton to minimization of RWC across the system.

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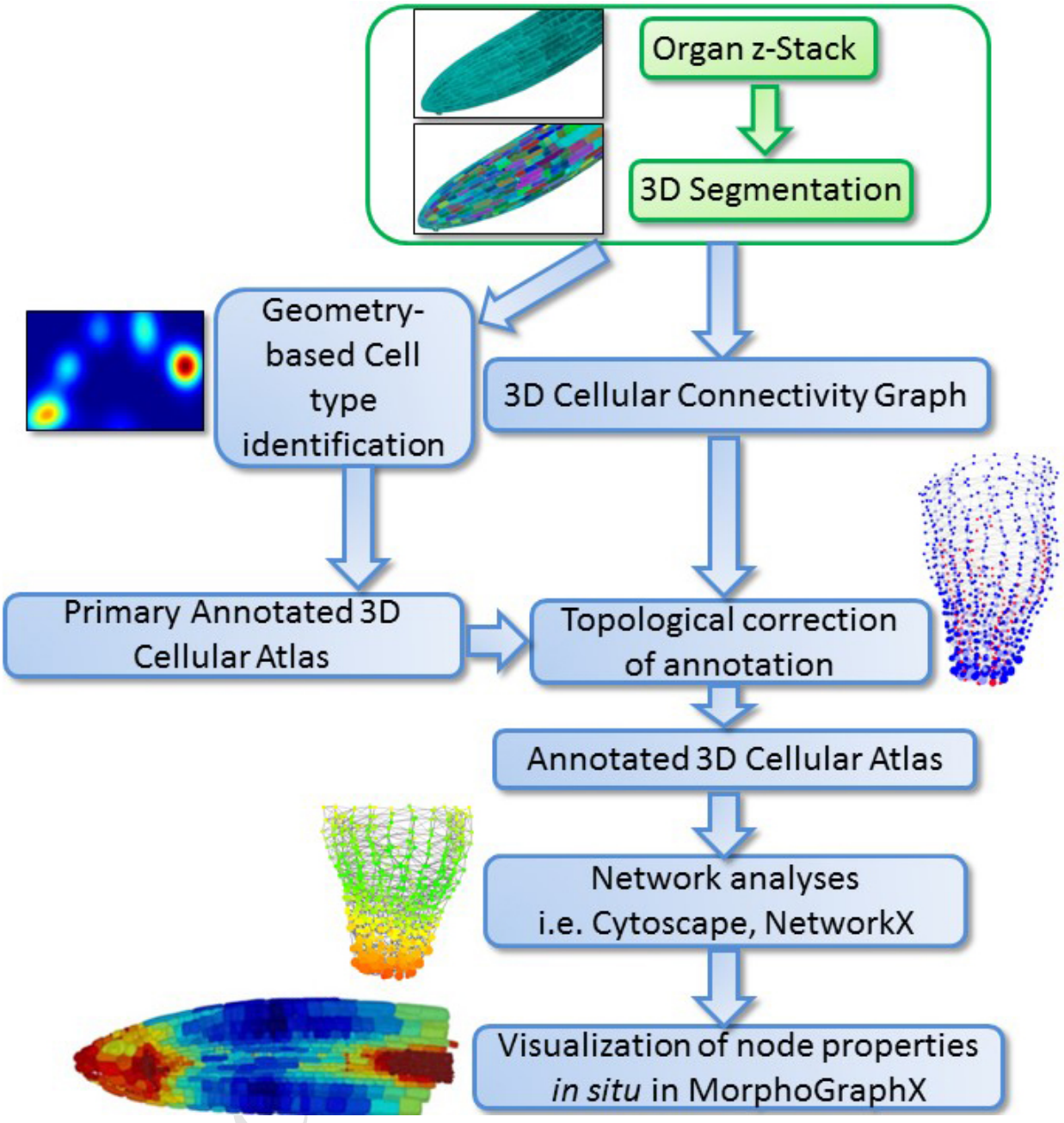
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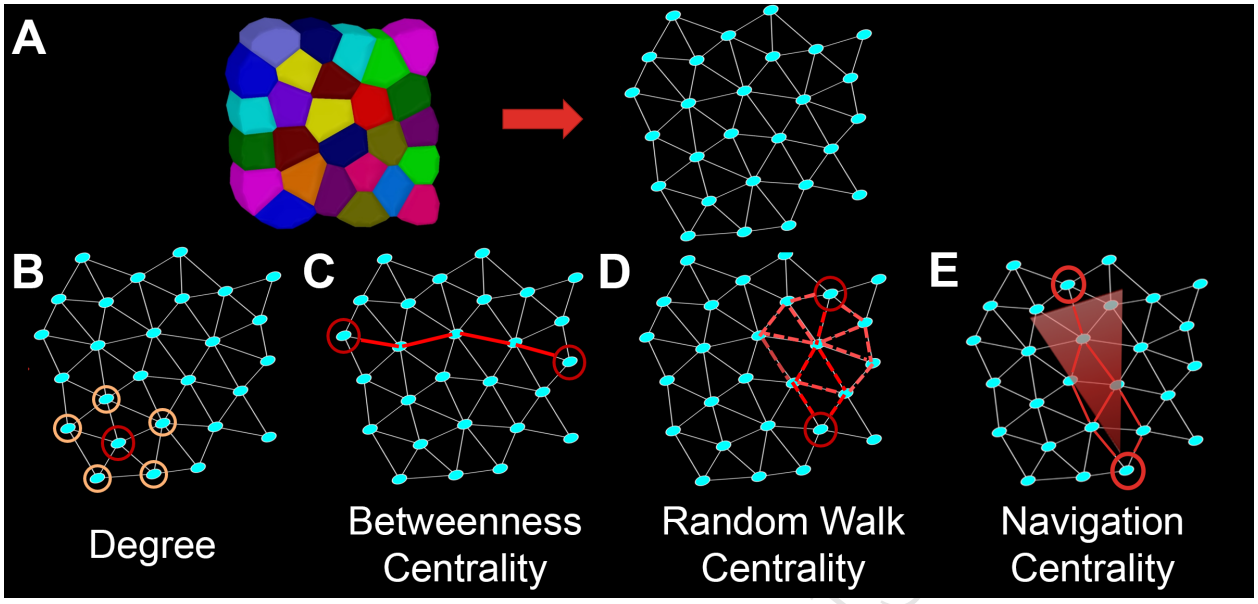

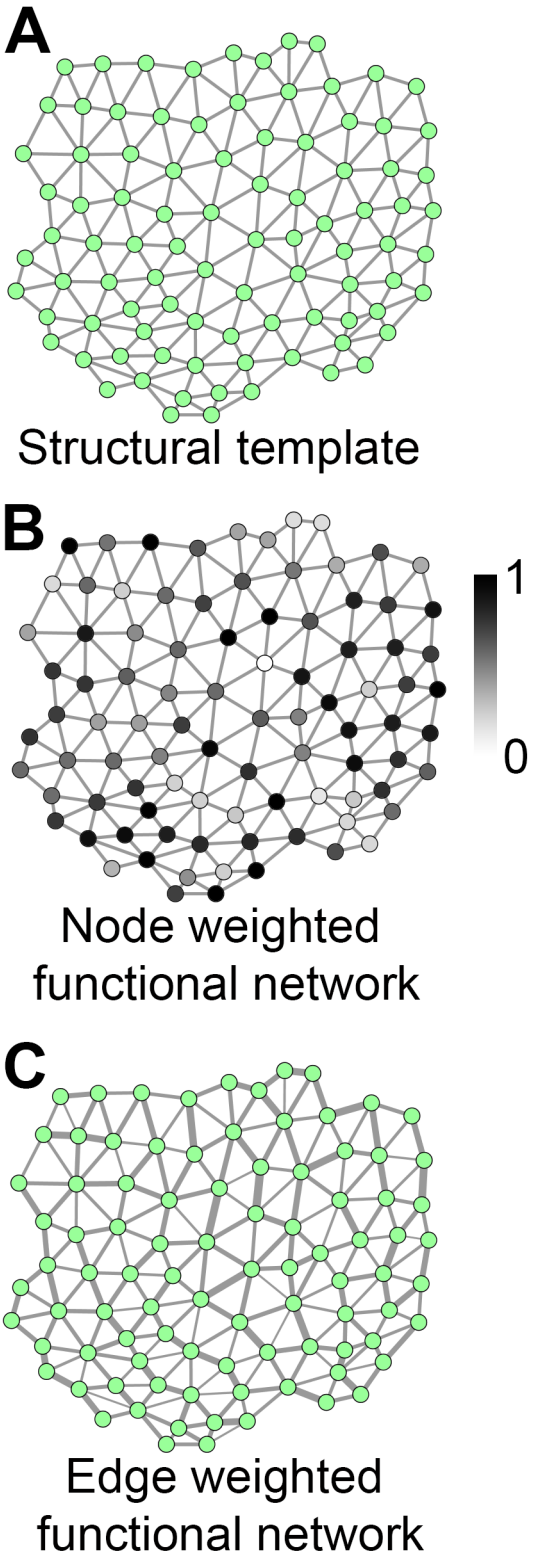

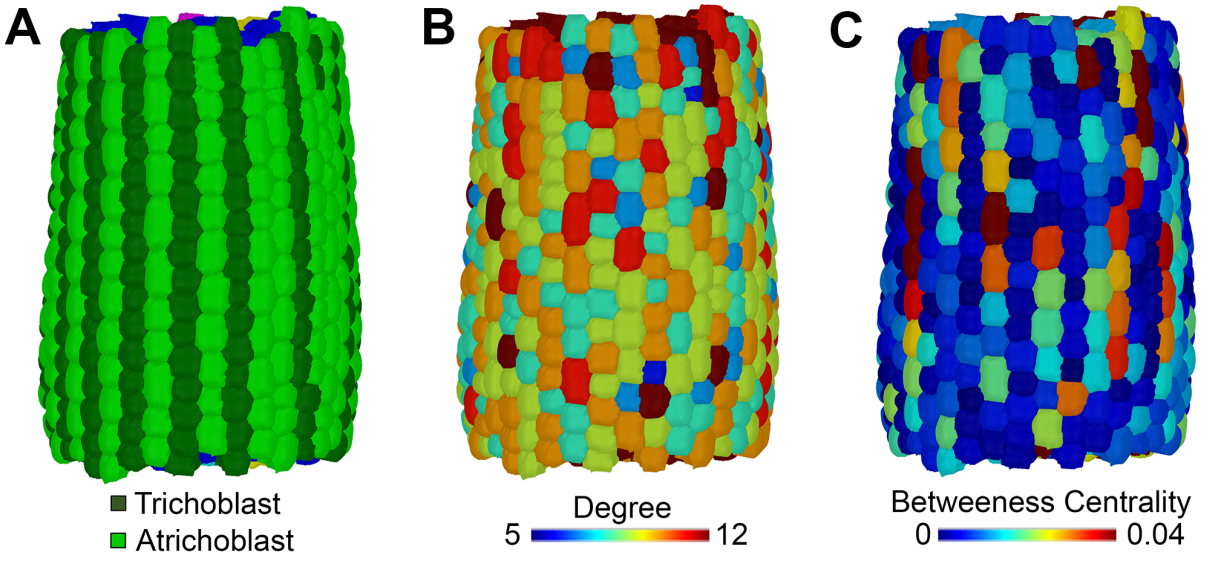
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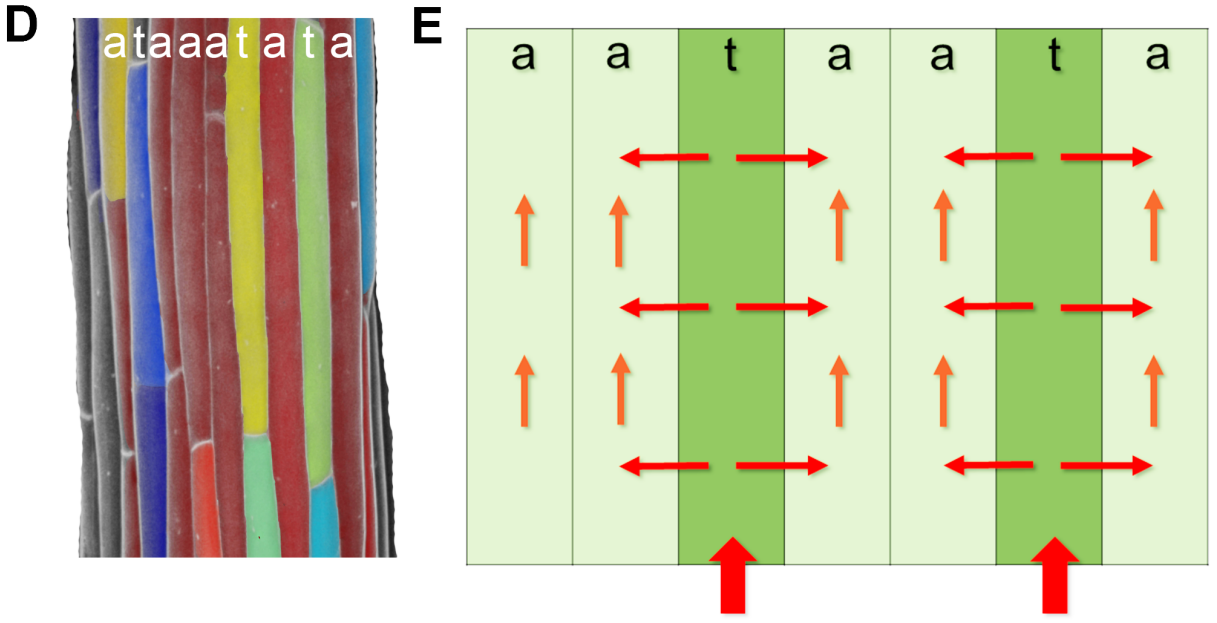
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